Experimental report

19/12/2016

Proposal: 9-10-1447 **Council:** 4/2015

Title: A new platform of Polymeric Nanocapsules with tunable morphology

Research area: Chemistry

This proposal is a new proposal

Main proposer: Cecile DREISS

Experimental team: Laura Marcela FORERO RAMIREZ
Daniela RIDOLFI
Cecile DREISS

Local contacts: Isabelle GRILLO

Samples: nanocapsules (Mygliol 810, PMMA, dextran)

Instrument	Requested days	Allocated days	From	To
D22	2	0		
D11	2	0		
D33	0	2	19/09/2015	21/09/2015

Abstract:

Nanocapsules (NCs) are nano-vesicular systems exhibiting a core-shell structure, particularly promising as carriers for drug delivery applications. The drug release profile is correlated to their morphology, particularly their shell thickness. However, very little is known about the detailed microstructure of polymeric NCs, in particular their shell thickness. The aim of this proposal is to elucidate the structure of a novel platform of NCs intended for the delivery of anti-cancer agents. The NCs comprise a biocompatible oily core Miglyol 810, a poly(methyl methacrylate) inner shell and a natural polysaccharide (dextran) as hydrophilic coverage. These have been prepared by the combined use of Miniemulsion polymerisation, Reversible Addition Fragmentation Chain Transfer (RAFT) polymerization and a transurf (both transfer agent and stabilizer during the polymerisation). This unique combination of techniques affords improved control over the NCs morphology. Our objective is to correlate polymerization conditions with structural parameters (oily core size, shell thickness) and, in turn, link these to the release characteristics.

A new platform of Polymeric Nanocapsules with tunable morphology

DREISS Cecile[a]; FORERO RAMIREZ Laura M.[b,c]; GRILLO Isabelle[d]; NOUVEL Cécile[b,c]; RIDOLFI D M[a] and SIX Jean-Luc[b,c].

a) *Institut of Pharmaceutical Science, King's College London*
b) *Université de Lorraine, Laboratoire de Chimie Physique Macromoléculaire LCPM, FRE 3564, Nancy F-54000, France*
c) *CNRS*
d) *Institut Laue Langevin (ILL)*

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Background

Research in nanomedicine, and in particular the design of new drug delivery systems, has attracted increasing attention over the last decades, in particular because of their potential in cancer therapy[1]. Among the various types of nanocarriers, nanocapsules (NCs) are particularly promising. NCs are nano-vesicular systems exhibiting a core-shell structure, the core acting as a liquid reservoir for drugs and the shell as a protective membrane. The versatile nature of the inner core, their high encapsulation efficiency and the low amount of solid content make them extremely attractive. The release kinetics of an encapsulated drug can be controlled by adjusting the nature and thickness of the polymer shell, and also the polarity and the volume of the liquid core[2]. It is thus clear that the control of NC morphology - particularly their shell thickness - is crucial in defining the release profile. However, mainly due to a lack of suitable techniques to explore scales in the nano-range, very little is known about the detailed microstructure of polymeric NCs, in particular their shell thickness. SANS is probably the only technique which can provide this detailed structural understanding, and thus help establish robust relationships between NCs morphology and their application, which are currently lacking. There are only a handful of published SANS studies on NCs[3,4], one from our group[4].

Our objective here was to elucidate the morphology of a novel platform of NCs intended for the delivery of anti-cancer agents. These were prepared by the combined use of Miniemulsion polymerisation, Reversible Addition Fragmentation Chain Transfer (RAFT) polymerization[5] and a transurf (both transfer agent and stabilizer during the polymerisation); this unique combination of techniques affords improved control over NC morphology in term of coverage, shell structure and NC size, as well as high drug encapsulation efficiency in a single step[6], compared to traditional processes using nano-emulsion templates[6] or phase segregation[2].

NCs preparation and characterization

We have developed a platform to synthesize dextran-covered NCs by miniemulsion polymerisation from a modified polysaccharide as a reactive surfactant, using RAFT[5] technique to control the polymerization. To ensure the biocompatibility and biodegradability of these nanocarriers, Miglyol 810 (M810, a neutral triglyceride widely used in pharmaceutical formulations[7]), poly(methyl methacrylate) (PMMA) and dextran have been chosen as the oily core, inner polymeric shell and hydrophilic coverage, respectively. The

dextran transurf both stabilizes the miniemulsion where MMA polymerisation takes place and acts as a macromolecular RAFT agent, ensuring a controlled growth of the polymeric chains (Fig. 1). As a result, grafted copolymers are produced at the interface, and the resulting NCs are constituted by an inner oil core and an inner shell made of the PMMA grafts linked to the dextran outer shell, providing fine control on the morphology.

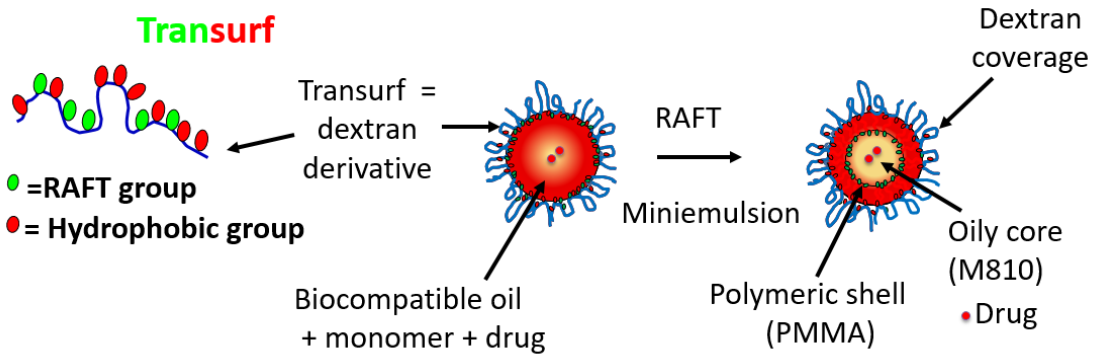

Fig. 1. NCs produced by RAFT miniemulsion polymerization from a dextran- based transurf.

NCs produced in this way were characterized by laser diffraction (~ 55 nm radius), zeta potential and colloidal stability studies. Zeta potential measurements allowed us to evidence the presence of the dextran outer shell and to estimate its thickness (~ 5 nm). In addition, this hydrophilic dextran coverage was dense enough to ensure colloidal stability of NCs at physiological ionic strength (0.148 M) and to provide protection against non-specific interactions with plasmatic proteins (BSA and fibrinogen). Besides, NCs displayed no toxicity to human monocytic THP-1 cells and could be considered as cytocompatible.

From a structural viewpoint, modulated DSC measurements enables us to measure M810 penetration in the PMMA shell (< 10wt%), thus providing precious information for the SANS data analysis. Preliminary CryoTEM measurements confirmed the core-shell structure and give an estimated shell of ~ 10 nm.

NCs morphology characterization by SANS

Different NCs samples were prepared in order to study the effect of four mainly parameters on NCs morphology:

- Effect of M810 content (25 and 50 v % relative to the monomer) of NCs having the same PMMA grafts length ($\bar{M}_{n\,PMMA} \approx$ 50 000 g/mol).
- Effect of PMMA grafts length ($\bar{M}_{n\,PMMA} \approx$ 50 000 g/mol and 21 000 g/mol) for NCs prepared with the same dextrane derivative (3.7 RAFT groups per 100 glucosidic units) and having the same M810 content (25 v % relative to the monomer).
- Type of dextrane derivative (3.7 and 7.5 RAFT groups per 100 glucosidic units).
- Interfacial polymerization vs. phase segregation. NCs were prepared with a dextran derivative having hydrophobic moieties but no RAFT groups instead of transurf, to compare our NCs platform to the simpler phase segregation approach.

In order to determine the parameters of interest without ambiguity, a method of continuous contrast variation was used, following the method used previously in a successful study[3], but improving it with deuterated MMA, which was expected to provide a much higher contrast between core and inner shell, and then inner shell and dextran layer. H_2O/D_2O composition was continuously varied (5 contrasts) to alternately contrast-match the oil core ($0.30x10^{-6}Å^{-2}$ = 88% H_2O), the inner shell ($9.77x10^{-6}$ $Å^{-2}$, or $6.81x10^{-6}$ $Å^{-2}$ by taking into account 10% M810 = 100% D_2O) and the outer dextran shell ($1.06x10^{-6}$ $Å^{-2}$ = 78% H_2O) + two intermediate contrasts (60 and 75% H_2O) and obtain a set of data that can be fitted simultaneously with the same set of parameters, thus separating the influence of size distribution from the influence of the form factor[3]. A set of 40 experiments were performed.

The analysis of the data collected is still in progress. This work is expected to complete the NCs morphological characterization and will be ideally published next year.

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